

How Does Selfing Affect the Pace and Process of Speciation?

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Surprisingly little attention has been given to the impact of selfing on speciation, even though selfing reduces gene flow between populations and affects other key population genetics parameters. Here we review recent theoretical work and compile empirical data from crossing experiments and genomic and phylogenetic studies to assess the effect of mating systems on the speciation process. In accordance with theoretical predictions, we find that accumulation of hybrid incompatibilities seems to be accelerated in selfers, but there is so far limited empirical support for a predicted bias toward underdominant loci. Phylogenetic evidence is scarce and contradictory, including studies suggesting that selfing either promotes or hampers speciation rate. Further studies are therefore required, which in addition to measures of reproductive barrier strength and selfing rate should routinely include estimates of demographic history and genetic divergence as a proxy for divergence time.

Speciation rates vary considerably among lineages, but the sources of this variation are still poorly known (Coughlan and Matute 2020). One factor that may affect the pace as well as the process of speciation is mating system, which ranges from obligately outcrossing to predominantly

selfing in many groups, including plants and animals (Igc and Kohn 2006; Jarne and Auld 2006; Billiard et al. 2012; Heesch et al. 2021). There is a long discussion about the possible roles of mating systems in speciation, especially in plants (Darlington 1939, 1956; Stebbins 1950; Grant

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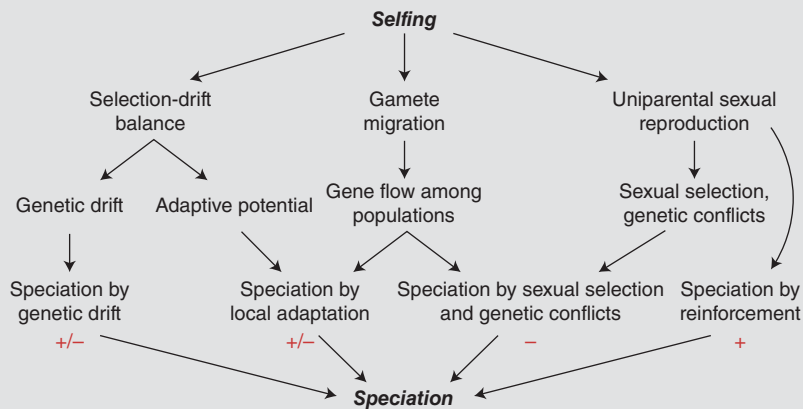
1956, 1971; Baker 1959). Early work, which was conducted in the framework of the biological species concept, mainly considered the effects of mating system on gene flow and interbreeding (Baker 1959; Grant 1971; Levin 1971). However, Baker (1959) pointed out that reproductive modes can affect the way species are delimited by taxonomists: Highly selfing species exhibit more discrete variation among populations than outcrossers, and this can lead to splitting into different species. Grant (1971) also noted that “microspecies” are more frequently recognized in selfers, but emphasized that there is always residual outcrossing, ensuring that the biological species concept remains applicable to selfers.

Beyond species concepts, two main issues have long been addressed and later revisited in a more formal framework. First, shifts in mating systems, usually from outcrossing to selfing, were early considered as a speciation mechanism and even a separate type of prezygotic isolating barrier (Levin 1971). This is because selfing automatically reduces gene flow from outcrossing populations to a degree corresponding to the selfing rate. Coyne and Orr (2004) later questioned “mating system isolation” as a separate isolating barrier and argued that a new selfing lineage does not constitute a separate biological species as gene flow would be equally restricted within the new lineage itself. However, this argument only considers the selfing taxon as the maternal parent, and the extent to which a selfing mating system will restrict pollen-mediated gene flow to species with other mating systems will depend on several factors, including pollination syndrome, levels of pollen production, and the timing of selfing (Martin and Willis 2007; Wright et al. 2013; Brys et al. 2016). In addition, Coyne and Orr’s (2004) main example was that individuals of a completely selfing lineage do not interbreed and therefore are not biological species. We consider this as overly black and white, as selfing rates rarely reach 100% (Grant 1971; Vogler and Kalisz 2001; Goodwillie et al. 2005). It is also not the same scenario as when a new partially selfing lineage evolves, and there is interbreeding within the lineage. Furthermore, the evolution of selfing not only leads to genetic isolation, but also to conspicuous changes in mor-

phological characters (the selfing syndrome; Sicard and Lenhard 2011) that can contribute to reproductive isolation (RI) (Levin 1971). Under this view, speciation can be considered as a side effect of the evolution of selfing, but it has also been proposed that selfing can be selected as a reinforcement mechanism (Antonovics 1968; Grant 1971; Epinat and Lenormand 2009; Rausher 2017). Second, it was recognized that selfing frequently is associated with polyploid speciation (Grant 1956; Stebbins 1957; Baker 1959), as it facilitates the establishment of newly formed polyploids by avoiding the selective disadvantage of mating with the possibly many surrounding diploid parents (i.e., avoiding minority cytotype exclusion; Levin 1975; Rodriguez 1996; Rausch and Morgan 2005). Overall, this led to the idea that sympatric speciation can be easier under selfing because it allows coexisting populations to remain genetically isolated and to diverge from one another (Grant 1971; Levin 1971).

In addition to its impact on prezygotic isolation, selfing affects key population genetics parameters such as homozygosity, effective population size, effective recombination rate, selection efficacy, and sexual and genetic conflicts (Box 1; Burgarella and Glémin 2017). It is reasonable, therefore, to expect that selfing can influence the accumulation of postzygotic incompatibilities, but this has largely been overlooked. Noting that variation in number or structure of chromosomes among closely related species tends to be more frequent in annual selfers than perennial outcrossers, Grant (1971) suggested that mating system could explain associations between reproductive barriers and life form, but without proposing a clear mechanism. Stebbins (1957) proposed that following hybridization between two partly incompatible species, selfing may favor the formation of “microspecies” by allowing differential sorting of compatible genetic combinations. In contrast, such compatible combinations are unlikely to be stable in outcrossers due to recombination. However, Stebbins did not discuss whether selfing could affect the initial accumulation of incompatibilities, which has been modeled only recently (Marie-Orleach et al. 2022).

The potential impact of mating system on speciation has also been integrated into a more

BOX 1. MAIN ROUTES BY WHICH SELFING MAY AFFECT SPECIATION

1. **Speciation by genetic drift.** Selfing increases genetic drift in two ways. First, mating is not random, and non-panmictic populations have reduced effective population size (halved in a fully selfing population compared to an outcrossing population with random mating; Pollak 1987; Nordborg 2000). Second, selfing makes genomes more homozygous, and recombination is thus less efficient at breaking up linkage between loci. Linkage disequilibrium is therefore more prevalent in highly selfing populations, reducing the efficacy of selection (Hill and Robertson 1966; Felsenstein 1974) and shifting the selection-drift balance toward more genetic drift. Genetic drift can lead to the accumulation of genetic incompatibilities in geographically isolated populations. Theoretical studies predict that selfing populations accumulate underdominant mutations, compensatory mutations, and Bateson–Dobzhansky–Muller (BDM) incompatibilities more readily than outcrossing ones, because low heterozygosity and recombination limit the production of unfit “intermediate” genotypes lying in fitness valleys (Charlesworth 1992; Marie-Orleach et al. 2022). However, genetic drift can also lead to the accumulation of weakly deleterious mutations and decrease mean population fitness (drift load), which can be restored through hybridization (the heterosis effect; Lynch 1991; Whitlock et al. 2000; Glémin et al. 2003). This may promote gene flow (Ingvarsson and Whitlock 2000) and hinder the build-up of reproductive isolation (RI), but the magnitude of this effect might be limited (Bierne et al. 2002).
2. **Speciation by local adaptation.** Selfing affects the adaptive potential of populations as well as gene flow among them, which may promote or hamper speciation by local adaptation (i.e., ecological speciation). In outcrossing populations, beneficial mutations are more likely to get fixed and contribute to local adaptation when they are dominant, because they are more exposed to selection in heterozygotes (Haldane’s sieve; Haldane 1927). In selfing populations, the high level of homozygosity combined with the lower efficacy of selection erases this dominance effect, making the fixation of beneficial mutations more likely when they are recessive but less likely when they are dominant (Charlesworth 1992; Ronfort and Glémin 2013). Moreover, linked selection reduces the efficacy of selection and the adaptive potential of selfing populations (Hartfield and Glémin 2016). Consequently, selfing populations inhabiting different environments may be expected to adapt and diverge more slowly than outcrossing ones. On the other hand, recent theoretical work predicts that even in the face of local adaptation, BDM incompatibilities may accumulate faster in selfing populations (Marie-Orleach et al. 2022). This is because reduced genetic shuffling in selfing populations facilitates the fixation of BDM incompatibilities. In addition, selfing populations may diverge and build up RI more easily than outcrossing populations due to reduced gene flow caused by less efficient gamete migration (Hodgins and Yeaman 2019; Trickovic and Glémin 2022).

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3. **Speciation by sexual selection and conflicts.** Sexual selection and conflicts can promote speciation in outcrossing species (Parker and Partridge 1998; Ritchie 2007; Gavrilets 2014; Janicke et al. 2018) but not in selfing species (Greeff and Michiels 1999; Ting et al. 2014; Cutter 2019). For instance, in outcrossing species, sexual conflicts may foster the coevolution of female traits spreading maternal resources equally over all embryos and of male traits that increase maternal provision only toward own sired offspring at the expense of unrelated embryos (Haig and Westoby 1989; Sandstedt and Sweigart 2022). However, such conflicts are irrelevant in selfed embryos (Brandvain and Haig 2005; Raunsgard et al. 2018), and sexually antagonistic coevolution of male and female traits underpinning maternal provisioning may thus foster population divergence and speciation only in outcrossers (Baack et al. 2015; Lafon-Placette and Köhler 2016).
4. **Speciation by reinforcement.** Selfing may itself be selected as a means to reduce gene flow between diverging populations (i.e., reinforcement; Ortiz-Barrientos et al. 2009; Castillo et al. 2016). Selfing seems to have evolved in *Minuartia* (formerly *Arenaria*) *uniiflora* to avoid costly hybridization with *M. glabra* (Fishman and Wyatt 1999). In fact, *M. uniiflora* is only selfing when the two species occur in sympatry, probably because heterospecific pollen transfer reduces *M. uniiflora* female fitness (Fishman and Wyatt 1999). In polyploid hybrids, selfing can be selected for to avoid genetic swamping by the parents (Barringer 2007). Moreover, the reduced pollen production typical of highly selfing species is expected to facilitate reinforcement (Rauscher 2017).

general macro-evolutionary perspective. Selfing was considered as a derived mating system (Darlington 1939, 1956; Stebbins 1950, 1957) that could foster speciation but also extinction, because of reduced adaptive potential (Stebbins 1957, 1974). This led to the hypothesis that selfing is an evolutionary dead end (Stebbins 1957), later more formally formulated as unidirectional evolution from outcrossing to selfing combined with negative net diversification rate (speciation rate minus extinction rate; Takebayashi and Morrell 2001; Igic and Busch 2013).

Taken together, mating systems have broader and deeper impacts than seminal works initially suggested and can modulate both the mechanisms and the pace of speciation, and ultimately the diversification process. In Box 1, we summarize recent theoretical work on this topic, and in the subsequent sections, we provide compilations of empirical data from crossing experiments and genomic and phylogenetic studies to assess the effect of mating systems on speciation. We tentatively conclude that several lines of evidence suggest that selfing lineages have higher speciation rates than outcrossers, but that this needs to be validated in further studies. Finally, we identify current knowledge gaps and possible future research directions.

SELFING AND EVOLUTION OF REPRODUCTIVE BARRIERS

To assess how reproductive barriers have been studied with respect to selfing, we compiled 58 plant studies reporting 319 crosses of selfing taxa (Supplemental Methods; Supplemental Tables S1 [in Supplemental methods] and S2). Among these 319 crosses, 44% were performed to examine prezygotic barriers and 67% to examine postzygotic barriers (note that some studies examined both types of barriers, and that findings varied from study to study; Table 1). In particular, we used this literature survey to elucidate what is known about the genetics of RI in selfing taxa, because selfing may influence the types of genetic incompatibilities that may accumulate (see above and Box 1). Below we discuss how selfing affects the development of pre- and postzygotic barriers and review our compiled empirical data on genetic incompatibilities in selfers (i.e., postzygotic barriers).

Prezygotic Barriers

In our literature survey, we found more crosses between taxa of different mating systems ($S \times O$; selfing \times outcrossing) than with selfing mating systems only ($S \times S$; 55% vs. 39%, respectively;

Table 1). In addition, 6% were crosses between selfing taxa and mixed mating taxa ($S \times M$; only reported in Supplemental Table S2 due to limited data). The higher percentage of crosses between selfing and outcrossing taxa probably reflects the attention selfing has received as the prezygotic barrier termed mating system isolation, which usually refers to a reduction in gene flow caused by the evolution of a new mating system (Coyne and Orr 2004). While this term also includes shifts to other mating systems such as agamospermy (asexual reproduction of seeds), selfing was originally considered a separate type of isolating barrier by itself (Levin 1971). Although it has been debated whether mating system isolation makes up a distinct isolating barrier (see above; Coyne and Orr 2004), selfing acts like other prezygotic barriers in that it can be reinforced if there is strong selection against the formation of hybrids (Box 1; Fishman and Wyatt 1999; Briscoe Runquist et al. 2014). Selfing can also be selected for in connection with local adaptation, as it prevents the break-up of locally adaptive traits by hybridization with neighboring populations (Box 1; Charlesworth and Charlesworth 1979). Selection for increased selfing has, for instance, been demonstrated in plant populations adapted to soils with high levels of toxic metals, as a means to avoid hybridization with populations adapted to unpolluted soils (Antonovics 1968; Lefèbvre 1970).

In a recent comparison between 12 isolating barriers in seed plants, mating system isolation was found to be the weakest (barrier strength from -0.05 to -0.29 ; Christie et al. 2022), but it should be noted that this result was based on only 10 taxon pairs. This study also found that mating system isolation was one of the most asymmetrical barriers (i.e., that the strength of RI depended on whether the selfing taxon served as the female or male parent). As selfing drives gene flow reduction, RI strength could be expected to be highest in the selfing taxon (e.g., Antonovics 1968; Levin 1971), but predicting the direction of asymmetry is not that straightforward (Tiffin et al. 2001; Brys et al. 2016). In fact, different levels of sexual/parental conflict in selfing compared to outcrossing lineages may cause opposite patterns. In outcrossers, many males can

compete to fertilize a female, which may create an evolutionary arms race between the sexes over resource allocation to the offspring (Box 1; Brandvain and Haig 2005). Such parental conflicts are less intense in selfers and highly inbred populations in which fertilization involves only a single or closely related males, and an outcrossing parent will therefore be “stronger” than an inbreeding parent in crosses between them. This is the basis for the weak inbreeder/strong outbreeder (WISO) hypothesis, which posits that the outcrossing parent will outcompete the selfing parent in crosses, potentially resulting in both pre- or postzygotic asymmetric RI (Brandvain and Haig 2005; Petré et al. 2023). At the prezygotic stage in plants, this may play out as selfers being outcompeted in pollen tube competition with outcrossers, termed the $SI \times SC$ rule (Murfett et al. 1996; Brandvain and Haig 2005). Pollen from a self-incompatible (SI; obligately outcrossing) species will more often germinate on the stigma of a closely related self-compatible (SC; potentially selfing) species than the other way around, and SC species are generally more receptive to foreign pollen, also from other SC species (Baek et al. 2016; Broz et al. 2017). Based on the WISO hypothesis, one possible explanation for the $SI \times SC$ rule is that the evolutionary arms race between the sexes in outcrossers will make their pollen tubes better at overcoming obstacles to fertilization (Brandvain and Haig 2005). Apart from parental conflict, it has also been shown that the increased drift load in selfers (Box 1) may lead to unidirectional introgression from outcrossing to selfing populations as a result of heterosis (Box 1; Busch 2006; Kim et al. 2018). Barriers are, however, not always found to be strongest in the outcrosser: a higher capacity for selfing (e.g., because of timing of selfing or anther-stigma positioning) may for instance counterbalance a “stronger” outcrosser (Ishizaki et al. 2013; Brys et al. 2014, 2016).

Even if one disregards mating system isolation as a separate prezygotic barrier, selfing may still promote prezygotic isolation by the development of selfing syndromes, the convergent evolution of traits such as small and inconspicuous flowers, loss of scent and nectar production, and reduced pollen production (e.g., Sicard and

Table 1. Genetics of reproductive isolation (RI) in selfers based on a literature survey of 58 papers including 319 diploid plant crosses

Studies	S × S		S × O ^a		S × O ^a : Species crossed
	37.4%	S × S: species crossed	52.7%		
Tests prezygotic RI ^b	48.4%		38.3%		
Finds prezygotic RI	60.0%		77.6%		
Tests postzygotic RI ^b	61.3%		71.4%		
Finds postzygotic RI	67.1%		92.2%		
QTL crosses	12		41		
QTL action					
Dominant	6	<i>Arabidopsis thaliana</i> (1,2) <i>Draba nivalis</i> (3) <i>Medicago truncatula</i> (4)	2		<i>Mimulus parishii</i> (S) × <i>Mimulus lewisii</i> (O) (11); <i>Mimulus nasutus</i> (S) × <i>Mimulus guttatus</i> (O) (12)
Partial dominance	4	<i>A. thaliana</i> (1) <i>D. nivalis</i> (3)	2		<i>Mimulus: M. parishii</i> (S) × <i>M. lewisii</i> (O) (11); <i>M. nasutus</i> (S) × <i>M. guttatus</i> (O) (12)
Additive	5	<i>D. nivalis</i> (3) <i>M. truncatula</i> (4)	6		<i>Arabidopsis: A. thaliana</i> (S) × <i>Arabidopsis arenosa</i> (O) ^a (13) <i>Lycopersicon: Lycopersicon esculentum</i> (S) × <i>Lycopersicon hirsutum</i> (O) (14) <i>Mimulus: M. nasutus</i> (S) × <i>M. guttatus</i> (O) (12,15); <i>M. parishii</i> (S) × <i>M. lewisii</i> (O) (10) <i>Mimulus: M. nasutus</i> (S) × <i>M. guttatus</i> (O) (15) <i>Lycopersicon: L. esculentum</i> (S) × <i>L. hirsutum</i> (O) (14) <i>Minuartia uniflora: (S × O^a)</i> (16)
Partial recessive	0		1		
Recessive	3	<i>A. thaliana</i> (2,5)	1		
Underdominant	1	<i>D. nivalis</i> (3) <i>A. thaliana</i> (6)	2		
Epistatic interactions	12	<i>A. thaliana</i> (1,2,5) <i>D. nivalis</i> (3) <i>Oryza sativa</i> × <i>O. nivara</i> (7) <i>O. sativa</i> × <i>O. sativa</i> (8) <i>Panicum hallii</i> (9)	9		<i>Arabidopsis: A. thaliana</i> (S) × <i>A. arenosa</i> (O) ^a (13) <i>Lycopersicon: L. esculentum</i> (S) × <i>L. hirsutum</i> (O) (17) <i>Mimulus: M. parishii</i> (S) × <i>M. lewisii</i> (O) (11); <i>M. nasutus</i> (S) × <i>M. guttatus</i> (O) ^a (12,18); <i>Mimulus lanciniatus</i> (S) × <i>M. guttatus</i> (O) (19) <i>M. uniflora: (S × O^a)</i> (16)
TRD	4	<i>A. thaliana</i> (5) <i>D. nivalis</i> (3) <i>O. sativa</i> (10) <i>P. hallii</i> (9)	7		<i>Arabidopsis: A. thaliana</i> (S) × <i>A. arenosa</i> (O) ^a (12) <i>Lycopersicon: L. esculentum</i> (S) × <i>L. hirsutum</i> (O) (17) <i>Mimulus: M. nasutus</i> (S) × <i>M. guttatus</i> (O) ^a (12,15,18); <i>M. parishii</i> (S) × <i>M. lewisii</i> (O) (11)

Continued

Table 1. Continued

Studies	S × S 37.4%	S × S: species crossed	S × O ^a 52.7%	S × O ^a : Species crossed
BDM	9	<i>A. thaliana</i> (1,2,5) <i>D. nivalis</i> (3) <i>Panicum hallii</i> (9)	6	<i>Capsella grandiflora</i> (S) × <i>Capsella rubella</i> (O) ^{1a} (20) <i>Mimulus</i> : <i>M. nasutus</i> (S) × <i>M. guttatus</i> (O) (21) <i>M. uniflora</i> : (S × O ^a) (16) <i>M. uniflora</i> : (S × O ^a) (16)
Structural divergence	1	<i>D. nivalis</i> (3)	2	
Cytonuclear	2	<i>D. nivalis</i> (3) <i>O. sativa</i> (10)	2	<i>Mimulus</i> : <i>M. parishii</i> (S) × <i>M. lewisii</i> (O) (11) <i>M. guttatus</i> (O) × <i>M. nasutus</i> (S) (incl. backcrosses) (22)

Only 16 of 58 were QTL studies including information on the genetics of RI. Number of crosses is reported if not otherwise indicated, as a single study could include multiple crosses with different results. Percentages are given of the total number of crosses in the header, and the total number of S × S and S × O crosses in rows 1–4. Note: Numbers for QTL action indicate the number of crosses reporting a particular kind of QTL action and not the total number of QTLs. (QTL) Quantitative trait locus analysis, (TRD) transmission ratio distortion, (BDM) Bateson–Dobzhansky–Muller incompatibilities, (S) selfer, (O) outcrosser.

^aAlso includes crosses where the outcrossing taxon is the seed parent (outcrosser × selfer).

^bThe percentage of crosses that were performed to examine pre- and postzygotic barriers, respectively, and not including information on whether the result was positive or negative: (1) Bomblies et al. (2007); (2) Alcázar et al. (2009, 2014); (3) Skrede et al. (2008) and Gustafsson et al. (2014); (4) Clo et al. (2021); (5) Bikard et al. (2009); (6) Oakley et al. (2019); (7) Nguyen et al. (2017); (8) Matsubara et al. (2015); (9) Lowry et al. (2015); (10) Refimur et al. (2014); (11) Fishman et al. (2015); (12) Fishman et al. (2001, 2002); (13) Burkart-Waco et al. (2012); (14) Moyle and Graham (2005); (15) Fishman et al. (2014); (16) Fishman and Stratton (2004); (17) Bernacchi and Tanksley (1997); (18) Kerwin and Sweigart (2017); (19) Ferris et al. (2017); (20) Rebernick et al. (2015); (21) Fishman and Willis (2001); (22) Fishman and Willis (2006).

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Lenhard 2011). For example, a selfing population of *Abronia umbellata* has smaller flowers and 99% less floral volatiles than outcrossing, moth-pollinated populations of the same species (Doubleday et al. 2013). This difference will reduce the probability of crosses between them. The reduced pollen production in selfers can also reinforce RI when hybrids produce less pollen than the outcrossing parent (Rausher 2017). Finally, selfing may contribute to allopatric speciation, as selfing facilitates establishment after long-distance dispersal (Baker 1955). Selfing can also evolve at range edges if there are few opportunities for outcrossing, possibly leading to “budding speciation” (Busch 2005; Wright et al. 2013; Koski et al. 2017).

Postzygotic Barriers

Prezygotic barriers are often predicted to contribute more to total RI than postzygotic barriers (Rieseberg and Willis 2007; Christie et al. 2022). However, recent theoretical studies indicate that partial prezygotic isolation by assortative mating is unlikely to be effective unless there is some postzygotic isolation as well (reduced hybrid fitness; Irwin 2020; Irwin and Schluter 2022). Theoretical work predicts that selfing facilitates the accumulation of hybrid incompatibilities, especially those caused by underdominant and compensatory mutations (i.e., mutations resulting in heterozygote fitness reduction and fitness restoration after deleterious mutations, respectively; Box 1). However, few studies in our literature review included information on the genetics of RI (only 16 of 58 studies; Table 1). Only three of these studies demonstrated postzygotic isolation due to underdominant mutations (Table 1). There is accordingly limited experimental evidence for the fixation of underdominant mutations in selfers. However, it is possible that underdominant mutations have been overlooked. Such single-locus models of hybrid incompatibilities include crossing fitness valleys and have traditionally been viewed as an unlikely road to speciation (e.g., Mack and Nachman 2017).

Bateson–Dobzhansky–Muller (BDM) incompatibilities, on the other hand, arise from negative epistatic interactions at two or more

loci and do not require crossing fitness valleys to be established (Orr 1995; Cutter 2019). They arise when new mutations are brought to fixation in allopatric populations and cause deleterious epistatic interactions when later combined (Orr 1995; Cutter 2019). BDM incompatibilities were much more commonly reported in our literature survey (21 crosses referring to epistatic interactions; and 15 specifically referring to BDM incompatibilities; Table 1). They may evolve as a by-product of selection as well as by drift and are predicted to accumulate most rapidly in selfers due to reduced effective population size and higher linkage disequilibrium (Box 1; Marie-Orleach et al. 2022). In selfing populations, BDM incompatibilities may especially manifest as loss of different gene duplicates in allopatric populations due to genetic drift, which leads to a complete lack of function in hybrids (Lynch and Force 2000). Cytonuclear incompatibilities (i.e., hybrid incompatibilities caused by negative interactions between cytoplasmic and nuclear genomes) are also forms of BDM incompatibilities. Interestingly, cytonuclear incompatibilities are tied to parental conflicts over investment in male versus female function as the cytoplasm typically is maternally inherited (Johnson 2010; Caruso et al. 2012), and such incompatibilities are also asymmetric. As mentioned in relation to prezygotic RI, different levels of parental conflict may lead to incompatibilities in crosses between selfing and outcrossing taxa. In our literature review, only four studies reported RI because of cytonuclear incompatibilities. However, we note that cytonuclear incompatibilities may be underreported in the literature, as their identification requires laborious reciprocal crosses to test the role of the parental sex.

Strong intraspecific postzygotic RI has been demonstrated in the harsh Arctic environment, where selfing plant species are especially common. Several taxonomically well-defined, diploid plant species belonging to different families produce more or less sterile offspring when conspecific populations from different geographic areas are crossed (Fig. 1; Brochmann et al. 1993; Grundt et al. 2006; Gustafsson et al. 2022). One hypothesis is that high selfing rates lead to high drift load, resulting in the evolution of intrinsic postzygotic

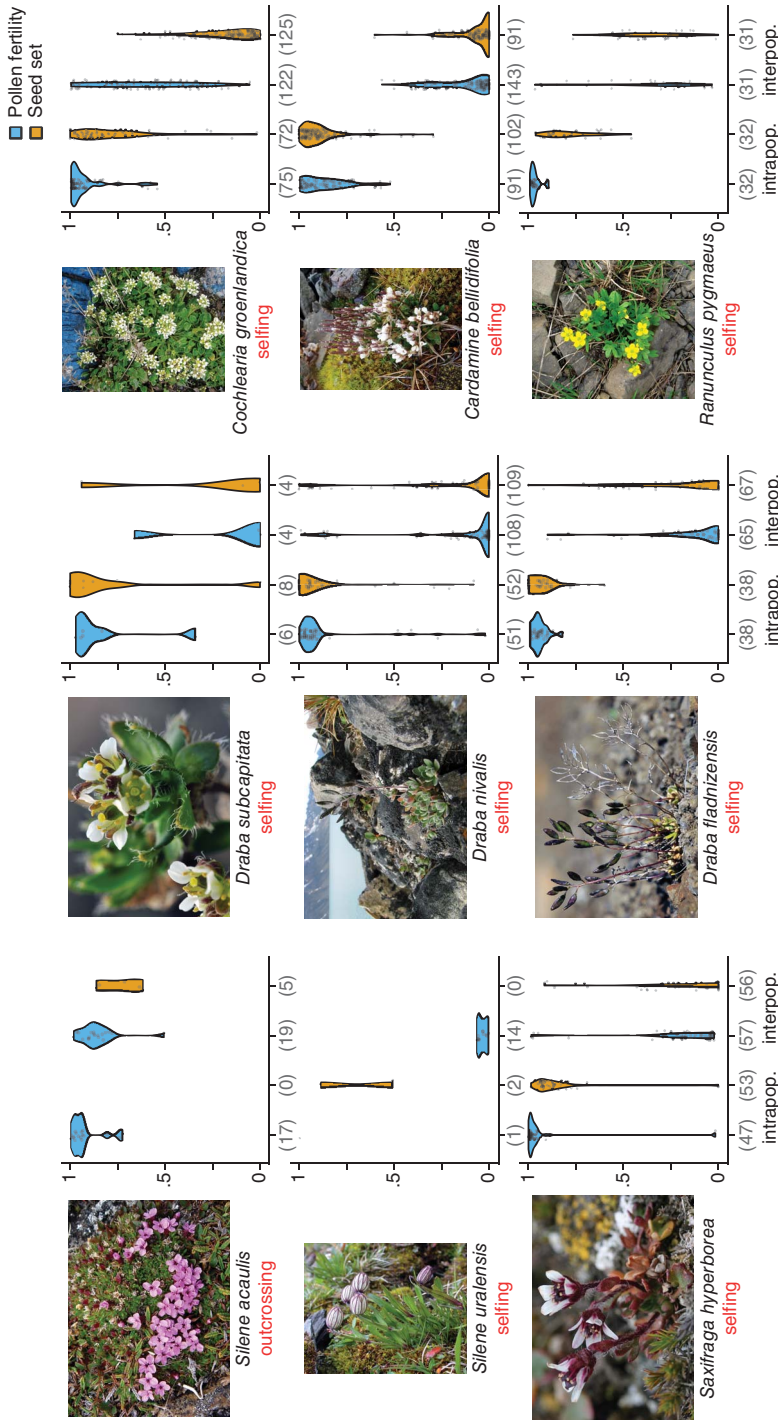


Figure 1. Rapid evolution of postzygotic reproductive isolation is widespread in selfing Arctic plant lineages. In extensive crossing experiments, Grundt et al. (2006) and Gustafsson et al. (2022) showed that taxonomically well-defined, diploid Arctic plant species produce sterile or partly sterile offspring when populations from different geographic areas are crossed, except in the only outcrosser they were able to cultivate and cross, *Silene acaulis* (top left). The vertical axes are proportions of fertile pollen grains (blue) or proportions of developed seeds relative to the total number of ovules (orange) for intrapopulation and interpopulation F1 individuals. Sample sizes (number of plants) are provided in parentheses below plots. See Grundt et al. (2006) and Gustafsson et al. (2022) for details. Photos were provided by Bjørn Erik Sandbakk (*Silene uralensis*, *Draba subcapitata*, *Draba fladnizensis*), Inger Greve Alsos (*Saxifraga hyperborea*), and Geir Arnesen (*Draba nivalis*) via svalbardflora.no.

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RI. Quantitative trait locus (QTL) mapping in the selfing Arctic crucifer *Draba nivalis* revealed a complex genetic architecture of RI, including underdominant loci most likely due to microchromosomal rearrangements as well as nuclear–nuclear and cytonuclear epistatic interactions between loci, indicating BDM incompatibilities (Table 1; Skrede et al. 2008; Gustafsson et al. 2014). Thus, one might expect such incipient species in the Arctic to be deeply divergent, but on the contrary, Gustafsson et al. (2022) estimated that they may have diverged even during the last few millennia. Taken together, both theory and observations on how selfing affects the evolution of RI suggest that postzygotic RI may accumulate faster in selfing than in outcrossing lineages.

EFFECTS OF SELFING ON THE PACE OF SPECIATION

How selfing influences the pace of speciation can be tested experimentally by assessing the strength of RI between diverging lineages. Here we discuss insights and limitations provided by two data sets on the strength of RI in selfing and outcrossing lineages.

First, to address the possible impact of different mating systems on the evolution of RI in plants, we compiled literature reports of F1 pollen fertility data (percent fertile pollen) from intraspecific crosses performed between populations. This data set spans 19 plant families and 90 diploid species (Fig. 2; Supplemental Table S3; Supplemental data set 1). We tested for the effects of mating system on F1 pollen fertility by building a phylogenetic linear model (using the R package `phylolm`; Ho and Ané 2014), which allows to account for phylogenetic nonindependence by informing the structure of the covariance matrix for the error terms. F1 pollen fertility was arcsine transformed. The phylogenetic tree was built with TimeTree (timetree.org). We found that mating system significantly predicts intraspecific F1 pollen fertility ($P = 0.010$; bootstrap likelihood ratio test with 1000 iterations). The pollen fertility of F1 individuals was on average lower in the selfing species (67.3 ± 5.0 ; mean \pm SE) than in the outcrossing species (87.4 ± 1.9), which may suggest that intrinsic

postzygotic isolation indeed evolves faster in selfing lineages (assuming that divergence times between populations are similar in selfing and outcrossing species; see below for limitations).

We included only diploid species in this data set because the build-up of RI may be affected by ploidy level, and because estimates of experimental intraspecific F1 pollen fertility appear to be rare in polyploid species. We note, however, that a striking pattern was observed in highly selfing Arctic species of *Draba*: Whereas intraspecific F1 pollen fertility was consistently low in the three diploid species examined (mean 12.3%, range 6.8–17.0), it was consistently high in the seven polyploid species examined (mean: 95.4%, range: 90.5–99.0; Brochmann et al. 1993; Grundt et al. 2006).

Second, Christie and colleagues (2022) recently published a study on the strength of pre- and postzygotic RI measured in 89 taxon pairs of seed plants. Their aim was to test how the relationship between barriers was influenced by factors such as life form, life history, geography, and mating system differences within taxon pairs (see above for a discussion of their findings on mating systems within taxon pairs). Here, we expanded their data set to test for the effect of mating system on RI among taxon pairs. We collected mating system information from the literature and categorized their species as outcrossers, mixed maters, or selfers (Supplemental Table S3). To obtain sufficient sample size, we lumped mixed maters and selfers (hereafter referred to as selfers)—and focused on pairs of outcrossers ($n = 38$) and selfers ($n = 15$). We computed the strength of prezygotic RI, postzygotic RI, and total RI by summing up all reproductive barriers using the equations in Sobel and Chen (2014), and ran phylogenetic linear models to test the effects of mating system (Supplemental Table S3 and Supplemental data set 2).

The most interesting insight from the data set of Christie et al. (2022) concerns the relationship between pre- and postzygotic RI. Most taxon pairs have strong pre- and/or postzygotic RI, but both are weak in four taxon pairs (Fig. 3). Our analysis shows that these four pairs are mixed maters or selfers: *Iris fulva* \times *hexagona*

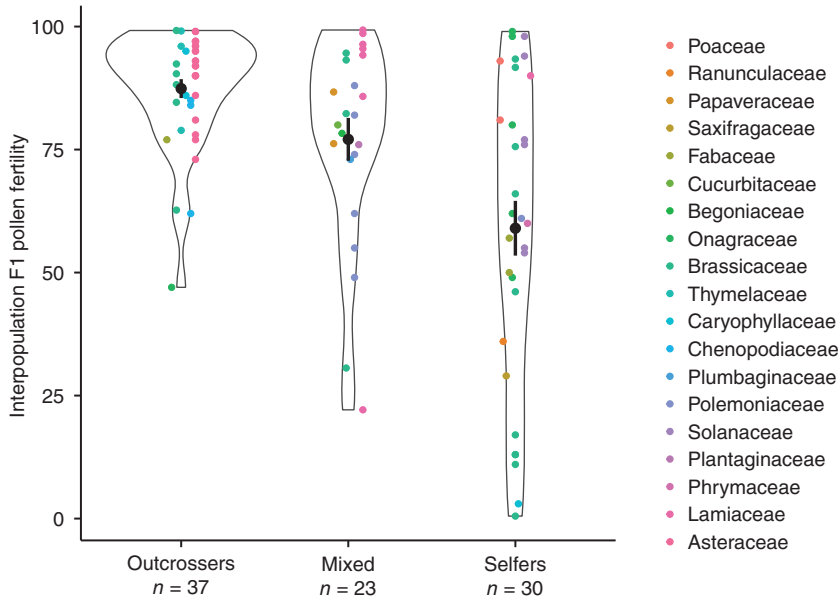


Figure 2. Pollen fertility of interpopulation F1 hybrids is lower in selfing than outcrossing plant species. Black dots with error bars represent means with standard errors. The figure is based on a compilation of experiments assessing the pollen fertility of F1 individuals produced from crosses between populations (see text for details).

(Emms and Arnold 1997), *Leavenworthia alabamica* × *crassa* (Koelling and Mauricio 2010), tetraploid × octoploid *Gladiolus communis* (Castro et al. 2020), and *Opuntia elata* × *retrorsa* (Fachardo and Sigrist 2020). It is interesting to note that despite weak RI, these pairs are reported as sympatric or parapatric in Christie et al. (2022). This pattern may indicate that selfing allows populations with weak RI to remain separated (i.e., speciation by reinforcement; Box 1), providing further support for classifying selfing as a prezygotic reproductive barrier.

The two data sets reported in this section provide important information on how selfing may influence the pace of speciation but are insufficient to provide a direct test. The critical limitation is that estimates of the genetic divergence between populations/species are lacking. It is possible, for example, that the selfing populations in Figure 3 produce less fertile F1 than the outcrossing ones simply because they are genetically more divergent. Thus, we strongly advocate that future studies should measure genetic divergence between populations and species used in experimental crosses.

MATING SYSTEMS AND THE DIVERSIFICATION PROCESS

The diversity of mating systems in flowering plants offers great opportunities to compare the macroevolutionary patterns of selfers and outcrossers, because systems preventing selfing have evolved and repeatedly been lost. Phylogenetic trees including species that differ in selfing rate can provide key information to assess the effect of selfing on the pace of speciation and more generally on macroevolutionary patterns. Because selfing lineages often are inferred to be restricted toward the tips of phylogenies (i.e., recently evolved), selfing has long been argued to be an evolutionary blind alley and selfing lineages to suffer from elevated extinction rates (Schoen et al. 1997; Takebayashi and Morrell 2001; Igic and Busch 2013). In this pervasive view, selfing would evolve within an outcrossing lineage, be evolutionary advantageous in the short run, and form a new lineage but go extinct in the long run. In agreement with the blind alley hypothesis, lower diversification rates are often reported in selfing lineages (e.g., Ferrer and Good 2012).

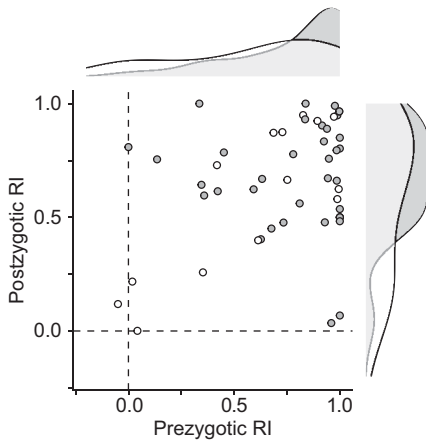


Figure 3. Species with weak prezygotic as well as weak postzygotic reproductive isolation (RI) are selfers. The graph displays the strength of RI in pairs of outcrossing species (gray circles) and selfing species (white circles) using the data set from Christie et al. (2022). The curves along the axes display the kernel density estimates of pre- and postzygotic RI in outcrossing species (gray) and selfing species (white). Pairs of selfers had weaker prezygotic RI (estimate \pm SE = -0.29 ± 0.11 ; $t = -2.7$; $P = 0.010$) and total RI (-0.26 ± 0.08 ; $t = -3.2$; $P < 0.002$) than pairs of outcrossers, but not significantly different postzygotic RI (-0.12 ± 0.10 ; $t = -1.25$; $P = 0.219$). For simplicity, we removed one outcrossing outlier with pre- and postzygotic RI of 0.64 and -0.83 , respectively.

Methods have been developed to estimate how traits influence rates of diversification, speciation, and extinction (state-dependent speciation and extinction [SSE] models; Table 2; Maddison et al. 2007; Helmstetter et al. 2023). In the plant family Solanaceae, self-incompatibility

(i.e., obligate outcrossing; SI) has been lost multiple times, leading to self-compatible (i.e., potential selfers; SC) lineages. Goldberg et al. (2010) found that the SC lineages had the lowest diversification rates, resulting from high rates of speciation but even higher extinction rates. Thus, in the Solanaceae, potential selfers speciate more but diversify less than obligate outcrossers. In addition, transition from SI to SC was about 10 times more likely to be associated with speciation than simple trait evolution within species (Goldberg and Igić 2012), supporting the idea that shift in the mating system can be an efficient driver of speciation. In other plant families, selfing has been found to have a nonconstant effect on speciation rates. In the Primulaceae and Onagraceae, loss of self-incompatibility was associated with a short-term burst of speciation that faded away with time (de Vos et al. 2014; Freyman and Höhna 2018). In contrast, selfing seems to slow down speciation rates in *Bulbophyllum* orchids (Gamisch et al. 2015).

Importantly, these studies have methodological limitations (Rabosky and Goldberg 2015), which may account for the inconsistent macroevolutionary effects of mating system found across taxa (Table 2). Moreover, phylogenetic trees can include consistent biases that artificially inflate or decrease species richness in selfing lineages relative to outcrossing ones. As noted above, selfing populations may be most easily considered as distinct taxonomic species because of more discrete genetic and phenotypic among-population variation (Baker 1959; Wright et al. 2013). On the other hand, outcrossing populations may be most easily considered as distinct

Table 2. Studies inferring rates of diversification, speciation, and extinction from phylogenetic trees using state-dependent speciation and extinction (SSE) models

Taxa	Diversification	Speciation	Extinction	References
<i>Bulbophyllum</i> genus	–	–	ns	Gamisch et al. 2015
<i>Mimulus</i> genus	–	–	ns	Castillo et al. 2016
Nematode phylum	+	+/ns	–/ns	Castillo et al. 2016
Polemoniaceae family	+/ns	+/ns	ns	Landis et al. 2018
Primulaceae family	– (nc)	ns (nc)	+	de Vos et al. 2014
Onagraceae family	–	+ (nc)	+	Freyman and Höhna 2018
Solanaceae family	–	+	+	Goldberg et al. 2010; Zenil-Ferguson et al. 2019

These macroevolutionary rates can be slower (–), faster (+), or not significantly (ns) different in highly selfing lineages. In addition, some studies reported nonconstant (nc) rates.

taxonomic species because traits emphasized by taxonomists (floral and sexual traits) evolve faster in outcrossing species.

Despite these limitations, statistical methods are improving, and it may appear that the most recent tend to confirm an effect of mating systems on diversification (e.g., Zenil-Ferguson et al. 2019). Nevertheless, more realistic models should be considered. In particular, the effect of mating system on diversification has only been analyzed under a binary framework (selfing vs. outcrossing, SC vs. SI) while selfing rates vary quantitatively. In particular, some models (e.g., Marie-Orleach et al. 2022) predict nonlinear effects with strong effect under very high selfing. Including more categories in analyses, in particular the difference between mixed mating and high selfing, would be more appropriate. Overall, we obviously need more robust phylogenetic studies to better understand whether the evolution of selfing consistently influences speciation rates across taxa or whether it only has idiosyncratic, context-dependent effects.

CONCLUDING REMARKS

The causes and consequences of mating system evolution are among the most studied topics in plant evolutionary biology. However, surprisingly little attention has been given to the impact of selfing on speciation. In this article, we have outlined many evolutionary parameters affected by selfing, predicted their impact on speciation, and assessed their alignment with empirical studies of speciation in selfing lineages. Prezygotic RI is generally weaker in selfing than in outcrossing species, probably due to reduced efficacy of selection in the former. However, sympatric species of selfers appear to avoid fusion at lower barrier strengths than in sympatric outcrossers, probably because selfing reduces gene flow within populations. Moreover, in accordance with theoretical predictions, we find that the evolution of hybrid incompatibilities appears to be accelerated in selfers during the earliest stages of divergence.

We found only limited evidence for some theoretical predictions. For example, in most cases we failed to find evidence that underdomi-

nant loci, such as chromosomal rearrangements, are important in the evolution of hybrid incompatibilities in selfers. However, this observation aligns well with a recent emphasis in the speciation literature on the role of natural selection in the establishment of large-scale inversions and translocations (Blackmon et al. 2019; Huang and Rieseberg 2020). Likewise, cytonuclear incompatibilities have only rarely been reported in genetic studies of selfing species, but this could be due to a paucity of studies that include reciprocal crosses rather than the rarity of this genetic architecture.

Going forward, we recommend that estimates of genetic divergence and demographic history become a standard component of studies of RI to ensure that variation in barrier strength is a consequence of biological differences rather than differences in divergence time or demography. Likewise, measurements of extrinsic postzygotic RI should, as far as possible, include multiple hybrid generations to better account for transient heterotic effects. We also encourage additional genetic mapping studies (including reciprocal mapping populations) of hybrid incompatibilities in selfing lineages to better test theoretical predictions about genetic architecture. More broadly, we hope our article highlights the richness of this topic and spurs further studies, especially in animals, of the theoretical predictions and partly surprising patterns described here.

AUTHOR CONTRIBUTIONS

L.M.O. and S.G. conceived the idea for the perspective. L.M.O., S.B., C.B., and S.G. developed and conceptualized the paper. L.M.O. and S.B. led and organized the work with the paper. L.M.O., C.B., and S.G. summarized the historical and theoretical background on selfing and speciation. S.B. led the work on reproductive barriers in selfers together with M.K.B., A.G., and L.H.R. S.B., M.K.B., and A.G. conducted the literature survey. L.M.O. led the work on the effect of selfing on the pace of speciation and conducted all analyses on this topic. L.M.O., A.G., A.L.S.G., L.H.R., and C.B. generated and collected the data set for these analyses. L.M.O., A.K.B., and S.G.

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led the work on mating systems and diversification. L.M.O. and A.L.S.G. made the figures in the paper. L.M.O., S.B., L.H.R., C.B., and S.G. wrote the manuscript with comments from all authors.

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